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Minireview

Dueling Activities of AIF in Cell Death versus Survival: DNA Binding and Redox Activity

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Apoptosis-inducing factor (AIF) was originally discovered as a mitochondrial protein that, like cytochrome c, is released into the cytoplasm during cell death. New evidence suggests, however, that a redox-active enzymatic region of AIF may be antiapoptotic while a DNA binding region is proapoptotic.

Elucidating mechanisms of cell death is of major importance to our understanding of both normal development and degenerative diseases. The discovery of the caspase family of enzymes represented a major breakthrough in this regard (reviewed in Cryns and Yuan, 1998). Recent findings, however, point to caspase-independent as well as caspase-dependent forms of apoptotic cell death in the nervous system and other tissues. One pathway to caspase-independent apoptosis involves the release of apoptosis-inducing factor (AIF) from mitochondria, in a sense paralleling the release of cytochrome c from mitochondria in caspase-dependent cell death (Liu et al., 1996). AIF was first identified and cloned by Guido Kroemer and colleagues (Susin et al., 1999; reviewed in Kroemer and Reed, 2000). Now a series of papers have appeared that further define the structure and function of AIF. The crystal structure of both murine (Maté et al., 2002) and human (Ye et al., 2002) AIF reveals two important regions, the first with oxidoreductase enzyme activity and the second representing a putative DNA binding site in a groove on the surface of the molecule (Figure 1). The redox catalytic region is structurally homologous to biphenyl dioxygenase (BphA4), a bacterial oxygenase-coupled NADH-dependent ferredoxin reductase that manifests a similar fold to the eukaryotic glutathione reductase family of enzymes. In functional assays, AIF acts as an NADH oxidase, i.e., accepting electrons from NADH. Importantly, the redox-active region is not essential for AIF's apoptogenic activity (Miramar et al., 2001; Joza et al., 2001). In living cells, AIF is located in the mitochondrial intermembrane space where its physiological function is not entirely clear. In dying cells, AIF relocates from the mitochondria to the nucleus where its DNA binding activity is thought to mediate chromatin condensation and large-scale DNA fragmentation (Susin et al., 1999; Ye et al., 2002).

Oxidoreductase Activity of AIF and Cell Survival

And that was the story until the latest chapter was published in *Nature* last week by a group led by Susan Ackerman at The Jackson Laboratory (Klein et al., 2002). Surprisingly, they found in an elegant series of experiments that the Harlequin (*Hq*) mutant mouse, which displays progressive degeneration of cerebellar and retinal neurons with aging, harbors a proviral insertion in the first intron of the *Aif* gene, leading to an 80% decrement in AIF expression. The startling finding came in experiments examining oxidative stress in mutant neurons. Cerebellar granule cells from the *Hq* mutant mice were more susceptible to peroxide-induced apoptosis than their wild-type counterparts. Conversely, retroviral transduction of wild-type AIF rescued the mutant cerebellar granule cells, leading the authors to suggest that AIF serves as a free radical scavenger to prevent apoptosis by a mechanism separate from its previously established proapoptotic function (Figure 2; Klein et al., 2002). An important alternative mechanism for the protective effect of AIF could arise if it normally plays a role in mitochondrial respiratory function and electron transfer. In that case, mutant AIF might produce a defect in mitochondrial respiration, increasing the production of reactive oxygen species and rendering neurons more susceptible to a wide range of insults, including oxidative stress (Suzuki et al., 1998; Wang et al., 2001). Damage to mitochondrial DNA might also be affected by mutant AIF. An interesting experiment to investigate the normal function of AIF would be to compare the respiratory pathways and electron chain coupling in isolated mitochondria from *Hq* mutant and wild-type mice.

The concept that oxidoreductase enzymes can participate in pathways affecting oxidative and nitrosative stress is not a new one (Dröge, 2002). However, the story does not appear to be a simple one in the case of AIF. Klein et al. posit that AIF could be a free radical scavenger and thereby ameliorate hydrogen peroxide-mediated apoptosis. It should be noted, in fact, that hydrogen peroxide (H_2O_2), while representing a reactive oxygen species, is not itself a free radical. It is possible that H_2O_2 may lead to subsequent generation of hydroxyl radical (·OH), although the mechanistic importance for this reaction in cell-based systems is still contested. Another way to detoxify hydrogen peroxide is to enzymatically scavenge it, e.g., convert it to water, similar to glutathione peroxidase, as suggested by Klein et al. (2002). However, AIF structurally resembles glutathione reductase and not glutathione peroxidase (Maté et al., 2002). Moreover, Kroemer, Susin, and colleagues have recently shown empirically, using in vitro biochemical assays, that AIF cannot by itself scavenge hydrogen peroxide. In fact, at least in vitro, AIF produces superoxide free radicals rather than scavenging free radicals (AIF catalyzes the net transfer of electrons from NADH to O_2, producing $O_2^{\cdot -}$) (Miramar et al., 2001). These findings would seem to contradict the hypothesized scavenging mechanism for the antiapoptotic function of AIF suggested by Ackerman and coworkers (Klein et al., 2002).

What, then, is the mechanistic basis for AIF's redox-mediated protection from peroxides? Many redox enzymes contain transition metal centers or critical thiol groups on cysteine residues (Stamler et al., 2001). These metals or cysteine residues can often participate in generating, scavenging, or cycling free radicals such as superoxide anion ($O_2^{\cdot -}$) and nitric oxide (NO·), or perox-

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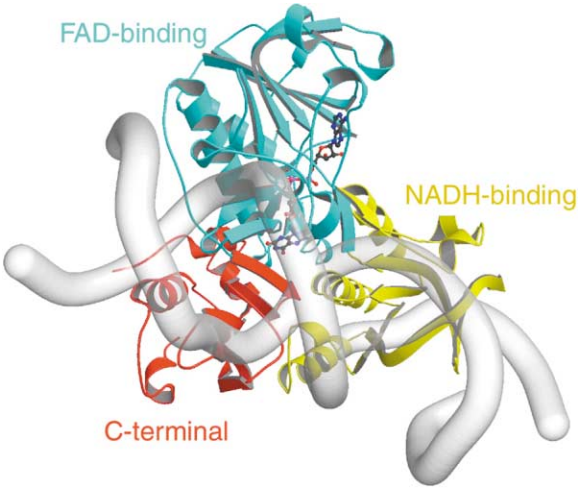

Figure 1. Three-Dimensional Crystal Structure of AIF

The redox-active site of human AIF demonstrates striking similarity to bacterial ferredoxin reductases with an N-terminal FAD binding domain (residues 128–262 and 401–480; FAD cofactor is shown as a stick model) and a central NADH binding domain (residues 263–400). However, the C-terminal domain (residues 481–608) is different from these bacterial reductases (Ye et al., 2002). A putative DNA binding groove extends from the FAD binding domain to the C terminus (docking of a DNA duplex to the structure is shown in gray).

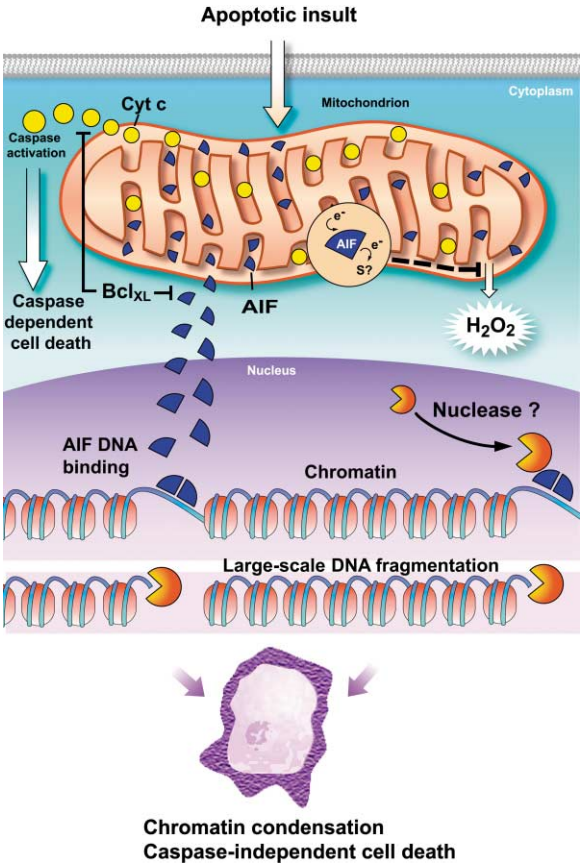

Figure 2. Model of AIF's Dual Function in Life and Death

AIF is normally confined to the mitochondrial intermembrane space, where it may act as an electron acceptor/donor with oxidoreductase activity. In response to some cell death stimuli in certain cell types, AIF, analogous to cytochrome c, is released from the mitochondria. However, AIF then translocates to the nucleus, where it binds DNA and triggers caspase-independent cell death. Bcl-x_L blocks both AIF and cytochrome c release from mitochondria. Loss of AIF and cytochrome c could contribute to disruption of the respiratory chain, increased oxidative stress (with potential generation of reactive oxygen species such as $O_2^{\cdot -}$ and H_2O_2), dissipation of the mitochondrial membrane potential ($\Delta\psi_m$), and decreased ATP synthesis. Similar to cytochrome c, the apoptogenic activity of AIF does not depend on its redox function. How exactly AIF mediates its apoptogenic effects remains unclear, but structural evidence suggests that AIF may bind DNA in a sequence-independent manner, perhaps as a dimer, which in turn may facilitate the recruitment of other factors such as nucleases. DNA binding appears to be required for AIF's apoptogenic function. In contrast, new work suggests that AIF protects cerebellar granule cell and retinal neurons from peroxide insult by an as yet unclear mechanism that could involve scavenging of H_2O_2 or maintenance of normal mitochondrial respiration. It is unknown if this action of AIF occurs in mitochondria, cytoplasm, or both.

ides (including H_2O_2). Indeed, AIF displays ~3 accessible thiol groups per molecule; importantly, however, thiol reagents do not affect the redox (NADH oxidase) activity of AIF, although they do block AIF's apoptogenic activity on isolated nuclei in cell free systems (Miramar et al., 2001). These biochemical findings suggest that the redox function of AIF is not mediated by thiols (but the apoptotic function may be since thiol reagents prevented this activity). Thus, cysteine thiols in the enzymatic region of AIF cannot be responsible for its postulated free radical scavenging ability. Similarly, AIF does not contain significant amounts of metals that could scavenge radicals. Rather, a flavin moiety (FAD) has been found to be noncovalently bound to AIF and is responsible for the electron transfer reactions from NADH (Miramar et al., 2001). So if cysteine residues and metal ions are not involved, what then is the mechanism for AIF protection in cells from peroxide-mediated insults?

One alternative that remains to be tested is that the NADH oxidase activity of AIF participates in vivo in a redox cycling or coupling pathway with other redox-active reactants, whereby electrons are transferred to detoxify free radicals or scavenge hydrogen peroxide. Redox cycling (alternate accepting and donating an electron) can serve as a protective mechanism. One potential clue for such a pathway comes from the other biochemical abnormalities noted in the mutant *Hq* cerebellar granule cell neurons: elevated catalase activity and increased total glutathione levels. Since catalase is a major scavenger of hydrogen peroxide, one might have postulated that these cells would be more resistant to peroxide rather than more susceptible, as observed empirically. On the other hand, the increase in catalase activity might be compensatory in mutant *Hq* neurons with limited AIF protein expression if AIF NADH oxidase activity normally participates in a redox cycle that ameliorates peroxide insult. Thus, a redox cycling or coupling scheme mediated in part by AIF could possibly account for the observed protection from peroxides, but potential redox partners of AIF other than NAD(P)H remain theoretical and unknown. Whatever the exact mechanism, the fact remains that exogenous and endogenous peroxide-mediated apoptosis in mutant *Hq* cerebellar neurons is abrogated by wild-type AIF (Klein

et al., 2002), but the jury is still out on the molecular basis for potential free radical or peroxide scavenging. Moreover, it would be useful to monitor free radical and peroxide production in the *Hq* cerebellar granule cells (in the presence and absence of forced AIF expression) after an oxidative or nitrosative insult to mitochondria is triggered, for example, by glutamate or *N*-methyl-D-aspartate (NMDA) (Ankarcrona et al., 1995). This experiment would determine if indeed free radical and/or peroxide levels are lowered by AIF overexpression.

The Relationship of AIF and PARP

Another part of the AIF puzzle in relation to oxidative stress stems from a recent and intriguing publication in *Science* from the laboratory of Valina and Ted Dawson at Johns Hopkins (Yu et al., 2002). In this case, NMDA-mediated death of mouse cortical neurons was associated with AIF release from mitochondria. NMDA and other forms of oxidative or nitrosative stress are also known to be associated with the activation of poly(ADP-ribose) polymerase-1 (PARP-1) (Zhang et al., 1994). PARP-1 is a nuclear enzyme that facilitates DNA repair after injury, transferring ADP-ribose to nuclear proteins with consequent NAD^+ depletion. To model this form of neuronal injury further, fibroblasts were exposed to H_2O_2 or a DNA-alkylating agent in order to trigger PARP activation and cell death. Specific AIF antibody injection into fibroblasts abrogated this form of cell death, and AIF peptides used as immunogens to make the antibodies (amino acids 151–170 and 181–200) reversed this protection. However, in their recent *Nature* paper, Klein et al. (2002) demonstrated that *Hq* mutant *Aif* cortical neurons, unlike cerebellar neurons, are no more sensitive to H_2O_2 than wild-type, warning therefore that each cell type can manifest different effects of AIF. Another possibility is that other forms of AIF or homologous gene products exist in different cell types (Wu et al., 2002). Hence, the comparison of actions of AIF in fibroblasts and neurons in the experiments by Yu et al. (2002) in *Science* should be viewed cautiously. Moreover, the requirement for AIF may not only be cell type-specific but also insult-specific. For instance, embryonic stem (ES) cells with *Aif* knockout display normal susceptibility to staurosporine, etoposide, azide, *tert*-butylhydroperoxide (*t*-BHP), anisomycin, and UV irradiation, but are resistant to menadione- and serum withdrawal-induced cell death (Joza et al., 2001). Thus, AIF's apoptogenic activity depends on the cell type and the cell death stimulus. Joza et al. (2001) also noted that targeted deletion of *Aif* in ES cells prevents embryoid body cavitation, representing the first wave of programmed cell death. However, such an early developmental effect is also consistent with a fundamental role for AIF in either energy metabolism (e.g., mitochondrial respiration, as discussed above) or in cell cycle control. Along these lines, Klein et al. (2002) observed evidence for abnormal cell cycle reentry followed by apoptosis in *Hq* mutant cerebellar granule cell and retinal neurons with decreased AIF activity; nonetheless, the delayed onset of these cell cycle abnormalities in aged *Hq* mutant mice argues that they are not a direct effect of AIF downregulation but perhaps due to oxidative stress.

One implication of the report by Yu et al. (2002) is that oxidative stress in cortical neurons leads to an AIF-dependent cell death pathway, but this was not observed by Klein et al. (2002) when they compared wild-type and *Hq* mutant *Aif* cortical neurons. Admittedly, the conditions of the two sets of experiments are not strictly comparable. One possibility for the difference between AIF antibodies but not the *Hq* mutation producing protection might be that the antibodies also inactivate an AIF-associated protein. In that case, an 80% knockdown in activity of AIF (as seen in *Hq* mice) might not affect the function of an associated protein while AIF antibodies might interfere by sequestration, yielding discordant effects. Alternatively, the remaining 20% of AIF expression in *Hq* cells might be sufficient to still mediate oxidative cell death in cortical neurons, whereas the AIF antibody experiments might abrogate the AIF apoptogenic activity. Another possibility is that AIF antibodies influence the redox activity in addition to—or instead of—the apoptogenic activity of AIF, since the immunizing peptides were against a domain in AIF responsible in part for the oxidoreductase activity. These examples are presented here merely to highlight the fact that we do not yet understand the dual mechanisms of AIF, especially with regard to function of the oxidoreductase activity and how AIF and associated molecules might affect redox signaling and cell survival.

A Role for NAD^+ Depletion to Facilitate AIF Free Radical Scavenging?

A final interesting dilemma with regard to the relationship of PARP and AIF concerns the depletion of NAD^+/NADH brought about by excessive PARP activation. In the absence of NAD(H), the normal oxidoreductase activity of AIF might be inhibited since NADH is a required cofactor. Does the depletion in NAD(H) result in mitochondrial dysfunction, injury, and AIF release (as postulated by Chiarugi and Moskowitz, 2002)? Additionally, could depletion of NAD^+/NADH result in AIF serving as a sink for free electrons? In other words, under conditions of pathological PARP activation, NAD(H) falls to very low levels. Hence, there is decreased NADH oxidase activity by AIF since there is little or no NADH available to be oxidized. One highly speculative idea is that under those specific conditions, AIF might accept electrons from a source other than NADH, such as a potentially toxic free radical, and thus act as a scavenger. Existing empirical data show that AIF can accept electrons from strong exogenous reductants (Miramar et al., 2001). If these or similar conditions hold, then the oxidoreductase activity of AIF would be antiapoptotic while its DNA binding activity would be proapoptotic, resulting in dueling functions of different regions of AIF with potentially different outcomes dependent on cell type and insult.

The Future of AIF Research

In summary, new studies suggest that under certain conditions AIF exhibits a "Jekyll and Hyde" dualistic nature, with an antiapoptotic function in the mitochondria via its oxidoreductase region and a separate proapoptotic action in the nucleus via its DNA binding region. Molecular, cell biological, and redox questions are critical to the evaluation of the bifunctional nature of AIF, and although great progress has been made during the past year, many questions remain unanswered. The normal function of AIF within mitochondria, which might contribute to respiration and electron transfer, must be studied. Since AIF has both nuclear and mitochondrial

localization sequences, it may have a dual role in dividing and nondividing cells. Perhaps AIF is released from mitochondria to decrease oxidative stress and limit calcium overload? In fact, cytosolic homologs of AIF, such as PRG3, might fulfill this role under normal conditions in cells exposed to unusually high levels of oxidative stress (Ohiro et al., 2002). It is therefore a great time for cell and molecular biologists to team up with structural and redox chemists to work out the details of the normal and pathological activities of AIF and related proteins. It will be important to identify the critical partners associated with AIF's redox function in mitochondria and its DNA binding properties in the nucleus. These processes are critical to the life and death of cells in response to oxidative and nitrosative stress experienced during normal aging and degenerative diseases.

Selected Reading

Ankarcrona, M., Dypbukt, J.M., Bonfoco, E., Zhivotovsky, B., Orrenius, S., Lipton, S.A., and Nicotera, P. (1995). Neuron *15*, 961–973.

Chiarugi, A., and Moskowitz, M.A. (2002). Science *297*, 200–201.

Cryns, V., and Yuan, J. (1998). Genes Dev. *12*, 1551–1570.

Dröge, W. (2002). Physiol. Rev. *82*, 47–95.

Joza, N., Susin, S.A., Daugas, E., Stanford, W.L., Cho, S.K., Li, C.Y., Sasaki, T., Elia, A.J., Cheng, H.-Y., Ravagnan, L., et al. (2001). Nature *410*, 549–554.

Klein, J.A., Longo-Guess, C.M., Rossmann, M.P., Seburn, K.L., Hurd, R.E., Frankel, W.N., Bronson, R.T., and Ackerman, S.L. (2002). Nature *419*, 367–374.

Kroemer, G., and Reed, J. (2000). Nat. Med. *6*, 513–519.

Liu, X., Kim, C.N., Yang, J., Jemmerson, R., and Wang, X. (1996). Cell *86*, 147–157.

Maté, M.J., Ortiz-Lombardía, M., Boitel, B., Haouz, A., Tello, D., Susin, S.A., Penninger, J., Kroemer, G., and Alzari, P.M. (2002). Nat. Struct. Biol. *9*, 442–446.

Miramar, M.D., Costantini, P., Ravagnan, L., Saraiva, L.M., Haouzi, D., Brothers, G., Penninger, J.M., Peleato, M.L., Kroemer, G., and Susin, S.A. (2001). J. Biol. Chem. *276*, 16391–16398.

Ohiro, Y., Garkavtsev, I., Kobayashi, S., Sreekumar, K.R., Nantz, R., Higashikubo, B.T., Duffy, S.L., Higashikubo, R., Usheva, A., Gius, D., et al. (2002). FEBS Lett. *524*, 163–171.

Stamler, J.S., Lamas, S., and Fang, F.C. (2001). Cell *106*, 675–683.

Susin, S.A., Lorenzo, H.K., Zamzami, N., Marzo, I., Snow, B.E., Brothers, G.M., Mangion, J., Jacotot, E., Costantini, P., Loeffler, M., et al. (1999). Nature *397*, 441–446.

Suzuki, H., Kumagai, T., Goto, A., and Sugiura, T. (1998). Biochem. Biophys. Res. Commun. *249*, 542–545.

Wang, J., Silva, J.P., Gustafsson, C.M., Rustin, P., and Larsson, N.-G. (2001). Proc. Natl. Acad. Sci. USA *98*, 4038–4043.

Wu, M., Xu, L.G., Li, X., Zhai, Z., and Shu, H.B. (2002). J. Biol. Chem. *277*, 25617–25623.

Ye, H., Cande, C., Stephanou, N.C., Jiang, S., Gurbuxani, S., Larochette, N., Daugas, E., Garrido, C., Kroemer, G., and Wu, H. (2002). Nat. Struct. Biol. *9*, 680–684.

Yu, S.-W., Wang, H., Poitras, M.F., Coombs, C., Bowers, W.J., Federoff, H.J., Poirier, G.G., Dawson, T.M., and Dawson, V.L. (2002). Science *297*, 259–263.

Zhang, J., Dawson, V.L., Dawson, T.M., and Snyder, S.H. (1994). Science *263*, 687–689.